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New Technologies in Single-Molecule Imaging

This project is focused on methods for imaging single events, and single fluorophores, in the living cell. Progress has been made over the past year on multiple fronts. First, we have improved our ability to selectively label specific proteins at defined loci. Second, we have improved our ability to detect and quantify the fluorescence of single fluorophores. Third, we have advanced a few assays that allow us to study the activity of single proteins in assays in vitro. Finally, we have started to migrate these assays to studies *in vivo.*

The labeled of proteins has been pursued with a few different strategies. We have advanced the use of inteins to allow us to regulate the protein splicing. Thus we can control, both spatially and temporally, the labeling of specific proteins. The conditional control has been achieved both with the use of small molecules as well as with light. The labeling of proteins has been further pursued by applying a technique developed elsewhere for using an acetyl CoA transferase which can recognize small peptide tags. The addition of this tag into the sequence of a protein results in the addition of an acetyl CoA to the site. However, it is possible to have the enzyme transfer a fluorophore of choice instead. There are a few advantages to this approach. First, when we insert the peptide tag of interest into our protein, we have used our modified fusion protein to replace the native wild-type protein in yeast. Thus, we can test if our modified tagged protein is fully functional (does it fully complement for the wild-type protein in function). Second, the tags are relatively small compared to fluorescent proteins. Third, we have more than one tag, thus two different fluorophores can be accurately and efficiently targeted to specific loci in the protein. Additionally, we have continued to pursue the use of quantum dots for labeled specific proteins and specific processes in cells.

For the imaging of single fluorophores, some of our effort has focused on technology: Improving our cameras, improving our optical paths. However, the bulk of the effort has been on developing software. This is driven by two motivations. First, we are studying single molecules, but we want to able to get detailed population histograms on the fluorophores we study. This requires automation to facilitate handling the large amounts of data. Second, not all fluorescent signals always appear the same. We believe that it is essential to establish an unambiguous criterion for selecting which photons that are detected are from our single molecules and which are the consequence of other unrelated phenomena. No preparation is 100% clean and there are often multiple sources of fluorescence. An automated selection criterion avoids any chance of investigator bias in the selection procedure.

Finally, we have made considerable progress in our assays. The two key assays are from our work on studying transport of single molecules in the cell and local signaling events on a fine subcellular level during apoptosis.